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(54) Title: FERMENTATION OF FRUIT PRODUCTS

(57) Abstract

A process for preparing a fruit product comprising the steps of: i) breaking and/or crushing the fruit; ii) adjusting the pH to 4.0 - 7.0, preferably to 5.5 - 6.5; iii) sterilising and/or pasteurising the broken/crushed fruit; iv) adding a culture of a Lactobacillus or Lactococcus strain producing when growing an extracellular polysaccharide at a rate of at least 0.8 g/l at 20 °C, a pH of 5.8 within 24 hours and a medium according to De Man, Rogosa and Sharpe (J. Appl.Bacteriol. 23: 130-135 (1960)) followed by fermenting; and v) pasteurising and/or sterilising the fermented fruit material. Preferably the fruit is from a plant of the family of the Solanaceae, more preferably from Lycopersicum esculentum (=tomato), or any cultivar thereof. Preferably the Lactobacillus/Lactococcus is selected from the group comprising Lactobacillus sake 0-1 (CBS 532.92), Lactobacillus paracasei (LMG 9193t1 and Lab 97) and Lactococcus lactis cremoris (LAB 338).

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FERMENTATION OF FRUIT PRODUCTS.

The invention relates to the fermentation of vegetable and/or fruit products, more in particular to the fermentation of crushed/broken fruits from plants of the family of the Solanaceae, in particular tomato, capsicum (paprika and/or pepper), Chili pepper and egg plant, preferably from Lycopersicum esculentum (=tomato), and any cultivar thereof.

This invention also includes the fermentation of mixtures of crushed/broken fruits as e.g. of tomato and pepper or mixtures of tomato and vegetables.

More in particular the invention relates to the preparation of tomato paste, tomato pulp, tomato juice or other tomato based products. Tomato paste is an important commercial product and is used as an ingredient for soups, sauces and ketchup. The largest part of the world tomato crop is processed into tomato paste and the present invention which relates inter alia to the preparation of tomato paste is therefore commercially important.

A typical tomato paste process comprises: tomatoes -> washing -> crushing/breaking -> heating -> pulping/sieving -> juice -> concentration -> paste, but many variations are known. Breaking can be effected at temperatures of about 90°-95°C (hot break) or at low temperatures of about 40°-60°C (cold break). Cold break favours degradation of cell wall material by pectolytic enzymes and the apparent viscosity, which is an important quality attribute, is increased. Adjustment of the Ph by addition of citric acid and degassing are steps which are often included to improve the end quality of the paste. The above processing steps cause physical, chemical and enzymatic

changes to occur in the tomato material which influence the rheological, other physical properties and organoleptic properties of the end product.

Enzymatic modification of tomato suspensions has been investigated (thesis F. W. C. den Ouden, Agricultural University Wageningen, The Netherlands 1995). The effects of pectin degrading enzymes caused tomato cells and particles to disintegrate into smaller particles and the values of rheological parameters of the suspension were generally, sometimes after an initial increase, found to decrease and moreover objectionable serum separation on top of fluid products increased which causes less consumer appeal. Serum separation is interrelated with thickness and a higher thickness tendency decreases serum separation. The tendency towards serum separation can conveniently be estimated on a laboratory scale by centrifuging the material.

Lebensm.-Wiss. u. Technol., 22, 65-67 (1989) discloses the preservation of whole ripe small ripe tomatoes (8-10g) by means of covering them with brine and subsequent subjecting them to lactic acid fermentation as to obtain a keepable fermented product that can be consumed in salad.

EP-A-0 308 064 (Kagome Kabushiki Kaisha) discloses to improve the flavour of a beverage based on tomato by lactic acid fermentation using particular lactobacilli strains. Thus tomato beverages are prepared with a "compound but unified flavour" by fermenting a processed tomato product, preferably together with a milk product with Lactobacillus bulgaricus and/or Lactobacillus helveticus. More preferably the fermentation is also carried out in the presence of Streptococcus thermophilus. It stated that only by using at least Lactobacillus bulgaricus and/or Lactobacillus helveticus that generation of so-called "off flavour" can be controlled during the lactic acid fermentation of a proces-

sed tomato product or its mixture and it is stated that beverages with a compound but unified flavour can be obtained efficiently.

It is clear from the prior art that lactic acid fermentation of tomato based products has been used in order to obtain keepable products. It is also clear that tomato products with an improved "unified" flavour can be obtained by lactic acid fermentation with very specific lactobacilli. At least some of these lactobacilli as e.g. L. brevis are heterofermentative and convert sugar into lactic acid, acetic acid, carbon dioxide and ethanol. Most lactic acid bacteria aim at the production of lactic acid and not at the production of extra cellular polysaccharides. However, fruit products like tomato juice, tomato paste and products derived therefrom, apple juice, etc usually also suffer from other disadvantages such as e.g. serum separation. Generally consumers like a keepable, thick, rich product showing no serum separation and having a good flavour. Food additives like colours, acidifying agents e.g. citric acid and thickening agents e.g. modified starch are, however, not generally appreciated. The present invention aims to provide fruit products with a favourable combination of the above features.

In a first embodiment of the invention a process for preparing an improved fruit product such as juice, paste or pulp is provided comprising the steps of:

i) breaking and/or crushing the fruit,

ii) adjusting the pH to 4.0 - 7.0, preferably to 5.5 - 6.5,

iii) sterilising and/or pasteurizing the broken/crushed fruit,

iv) adding a culture of a Lactobacillus or Lactococcus strain producing when growing an extracellular

polysaccharide at a rate of at least 0.8 g/l at 20°C, a pH of 5.8 within 24 hours in a medium according to De Man, J.C., M. Rogosa and M.E. Sharpe (J. Appl. Bacteriol. 23:130-135, 1960), followed by fermenting, and

v) pasteurisation and/or sterilisation of the fermented fruit material.

More preferable the rate of producing extracellular polysaccharide (EPS) mentioned above is at least 1.0 g/l, most preferably at least 1.2 g/l.

Breaking and/or crushing the fruit is conveniently carried out after first washing and blanching or scalding the fruit, the tomatoes are then broken and/or crushed using e.g. a chopper or vacuum crusher. There are the possibilities of a "hot break" or a "cold break" as set out above. The broken and/or crushed fruits may then be refined i. e. extracted or sieved to remove peels, seeds and possibly stems. However, it is also possible to carry out the refining step later in the process. Suitable equipment is e.g. an extractor of the screw type or of the paddle type. The juice obtained is then optionally deaerated and/or salted. The pH of the fruit mass is then adjusted to 4.0 - 7.0, preferably to 5.5 - 6.5 by the careful addition of a basic substance usually food grade sodium hydroxide. The exact pH value selected is usually determined by the optimal pH value for the growth of the particular Lactobacillus or Lactococcus strain to be employed. When it is intended to use e.g. Lactobacillus sake 0-1 (CBS 532.92) of which the optimal pH value is known to be 5.8 an initial pH value somewhat above 5.8 is selected so that the Lactobacillus grows well during the fermentation period. For other suitable Lactobacilli and/or Lactococci different values will generally apply.

Dependent on the nature of the Lactobacilli and/or Lactococci actual fermenting may take place under different

conditions. Again in the case of Lactobacillus sake 0-1 (CBS 532.92) fermenting takes preferably place at a temperature between 15 and 35°C and under conditions without forced supply of oxygen for a period of 5 - 30 hours. Generally the ranges of the fermentation temperature is somewhat wider viz. from 10 - 35°C anaerobic conditions may not be required. Fruits employed in the practice of this invention generally have a dry matter content below 10% (tomatoes 5-7.5%) of which about half consists of reducing sugars mostly D-fructose and/or D-glucose. Lactobaccilli and Lactococci convert these sugars when growing into lactic acid and polysaccharide. The percentage of soluble solids is conveniently expressed according to the Brix scale (i.e. calculated as sugar) and refractometers therefore often have in addition to the refractive scale a Brix scale.

After fermenting the Lactobacilli and/or Lactococci are inactivated usually by heat treatment i.e. by pasteurisation and/or sterilisation. Thereafter the fermented product may be deaerated and packed. Quite often packing takes place before pasteurisation/sterilisation. When aiming to produce a fruit paste as e.g. tomato paste concentration of the fermented liquid e.g. to a strength of around 7.5. Brix is desirable. Concentration is conveniently effected in tanks with heating coils or in vacuum pans.

In a preferred embodiment of the invention such a process is provided in which the fruit is from a plant of the family of the Solanaceae, preferably from Lycopersicum esculentum (=tomato), or any cultivar thereof. A particularly preferred group of tomato cultivars for the practice of this invention are the so-called "Pomodori" of Italy. For special effects it maybe desirable to use mixtures of e.g. tomatoes and paprika, or tomatoes and vegetables or

their juices, preferably at least 50 wt% of tomatoes, more preferably at least 80 wt% of tomatoes are used.

In another preferred embodiment of the invention the Lactobacillus or Lactococcus strain producing when growing an extracellular polysaccharide is selected from the group Lactobacillus sake 0-1 (CBS 532.92), Lactobacillus paracasei (LMG 9193t1 and Lab 97) and Lactococcus lactis cremoris (LAB 338). LMG and LAB are abbreviations which indicate that the strains have been deposited at collections kept at Ghent University, Belgium. The use of mutants including those obtained by DNA-recombinant technology or classical mutagenolysis derived from the above Lacobacilli and Lactococci which are functionally equivalents of those identified above as to EPS and lactic acid formation is also covered by the present invention. The above identified microorganisms form during growth not only lactic acid, but also polysaccharides which thicken the fruit mass. As far as e.g. Lactobacillus sake 0-1 (CBS 532.92) is concerned reference is made to Appl. and Environm. Microbiol., 6 (August 1995), pp. 2840-2844 in which inter alia the exopolysaccharide formed by Lactobacillus sake 0-1 which comprises glucose and rhamnose units is more fully identified. The use of a Lactobacillus or Lactococcus strain producing an extracellular polysaccharide containing rhamnose units is preferred inter alia because this may lead to particularly favourable flavour effect in the processed end product.

In a further preferred embodiment of the invention deaeration of the broken and/or crushed fruit is effected prior to pasteurisation and/or sterilisation.

In a further preferred embodiment of the invention sieving is effected prior to pasteurisation and/or sterilisation.

In a further preferred embodiment of the invention fermentation is carried out at a temperature between 10 and 50°, preferably 20 and 40°C in the absence of supplied oxygen or air.

In a further preferred embodiment of the invention prior to fermentation with the Lactobacillus or Lactococcus strain a saccharide, preferably sucrose is added to the broken and/or crushed fruit material. More preferably the amount of sucrose in the broken/crushed fruit material is adjusted to a level of 15-25 g/kg fruit material. Especially when using Lactobacillus paracasei (LMG 9193t1) the addition of sucrose is beneficial.

In a further embodiment of the invention the process from step ii) onwards is preferably carried out under aseptic conditions. Under these circumstances sterilisation to obtain the end product may be superfluous.

In a still further embodiment of the invention an enzymatic treatment of the fruit product is inserted between steps iv and v of the process, preferably using oxidoreductases such as peroxidases and/or glucose-oxidases.

Furthermore, the invention provides a fermented food product obtainable by a process as described above. These products compare favourably with those known sofar as to the properties mentioned above. More in particular they are improved as to serum separation, and thickness, and sometimes also as to colour and taste etc. Moreover their content of labelled food additives as e.g. thickening agents and citric acid can be minimised or their use even completely avoided.

The invention is further illustrated by the following examples. All parts and percentages in this specification and claims are taken on a weight basis unless otherwise indicated.

Example 1.

About 2 kg of fresh tomatoes (bought in a local supermarket) were stripped from stalks and washed with tap water. Subsequently the tomatoes were crushed for 3 min (at position 2-5) using a type Kenwood major (Kenwood Ltd, UK) electronic kneader / mixer. After crushing the seeds and skins were removed by centrifugation and sieving using a type Braun (Braun, Germany) household centrifuge and a laboratory test sieve type ASTM 11, 30 mesh (Endecots Ltd, UK) respectively, resulting in a tomato juice (suspension) having a dry matter content of 4.8%, a pH value of 3.93 and a Brix value of 4.5. After adjusting the pH value to 6.3 with food grade sodium hydroxide the juice was pasteurised (2 min - 100°C), cooled to 28°C and inoculated with Lactobacillus sake 0-1 (CBS 532.92) and to a starting cell concentration of 2 x 10^6 cells per gram of juice (determined as colony forming units). Subsequently the inoculated tomato juice was fermented and processed as described below in more detail in example 5.

Table 1. Effect of fermentation on viscosity and sensoric properties of tomato juice.

Sample	run time / (s)	Sensoric properties
Unfermented juice	4	n.d.
Fermented juice	6	excellent

Rheological measurements (run time):

To demonstrate the effect of fermentation on the rheological properties of tomato paste a fermented sample was compared with an unfermented sample in a standardised viscosity test using a type EZ™, equivalent "Zahn" viscosity cup (Gardco, USA), and which is said to exceed ASTM D4212, which cup has a diam. 5 mm orifice.

Example 2.

The same procedure as in Example 1 was used, however, after centrifugation the tomato paste was heated for 2 minutes at 98 - 100°C and concentrated to a Brix value of 7.0 using a type Buchi R-124 Rotavapor vacuum evaporator operating at 65°C and a pressure of 15 - 20 kPa.

Table 2. Effect of fermentation on serum development in tomato paste at x 540 g force (dm = 7.8 %).

Sample / centr. time	0 minutes	3 minutes	6 minutes	9 minutes
Unfermented	< 1	31	35	34
Fermented	< 1	3	8	11

Rheology according to method as described in example 1.
The organoleptic properties of the fermented product were excellent.

Example 3.

About 2 kg of fresh tomatoes (type Italian Pomodori, bought at a local wholesaler) were stripped from stalks and washed with tap water. Subsequently the tomatoes were crushed for 3 minutes (at position 2-5) using a Kenwood major (Kenwood Ltd, UK) electronic kneader/mixer. After crushing the seeds and skins were removed by centrifugation and sieving using a Braun (ex Braun, Germany) household centrifuge, resulting in a tomato paste having a pH value of 4.29 and a Brix value of 5.0. After adjusting the pH value to 6.3 with food

grade sodium hydroxide the paste was heated for 2 minutes at 98 - 100°C and concentrated to a Brix value of 7.0 using a type Buchi R-124 Rotavapor vacuum evaporator operating at 75°C and a pressure of 15 - 20kPa. Subsequently the tomato paste was pasteurised (2 min - 100°C) cooled to 28°C and inoculated with Lactobacillus sake 0-1 (CBS 532.92) and a starting cell concentration of 2 x 10^6 cells per gram of paste (determined as colony forming units). Thereafter the inoculated tomato paste was fermented for 24 hours at 28°C. After fermentation the tomato paste was pasteurised for 30 minutes at 80°C, cooled to room temperature and used for organoleptic - and rheological analysis as described in Example 5. The product according to the invention proved to have very good organoleptic properties.

Table 3. Effect of fermentation on serum development in tomato paste at x 540 g force (Brix value 7.1)

Sample / centr. time	0 minutes	3 minutes	6 minutes	9 minutes
Unfermented	< 1	37	39	40
Fermented	< 1	< 1	< 1	< 1

Rheology according to the method described in Example 1.

Example 4.

About 2 kg of fresh tomatoes (type Italian Pomodori, bought at a local wholesaler) were manually stripped from stalks and skins (after 1 minute immersion in boiling water) and washed with tap water. Subsequently the tomatoes were cut into 4 pieces and heated in a microwave oven (type Amano, 750W) for 10 minutes till the temperature reached 90°C. After heating the tomato pieces were processed into a paste using a Hobart N 50 (ex Hobart, Holland) kneader/mixer and sieved through a diam. 0.9-1.1 mm orifice using a type

Hobart 200S (ex Hobart, Holland) pilot sieving unit. After adjusting the pH value to 6.3 the tomato paste was further processed and fermented as described in the previous example (Ex. 3).

Table 4. Effect of fermentation on serum development in tomato paste at x 540 g force (Brix value 7.0)

Sample / centr. time	0 minutes	3 minutes	6 minutes	9 minutes
Unfermented	< 1	38	38	40
Fermented	< 1	< 3	< 8	< 9

Rheology according to the method described in Example 1.

Example 5.

Commercially available tomato paste (type Pummaro, ex STAR, Milan, Italy). Thus 100 g of Pummaro product was aseptically transferred into a sterile 300 ml size glass bottle and inoculated with 0.5% of a washed cell suspension of the lactic acid bacterium type Lactobacillus sake 0-1 (CBS 532.92) and to a starting cell concentration of 2 x 10^6 cells per gram (determined as colony forming units).
Before inoculation the pH of the tomato matrix was adjusted to a value of 6.4 +/- 0.1 by mixing in approx. 0.5% food grade NaOH (10.8 mol/l).
Subsequently the inoculated tomato paste was fermented for 24 hours at 28°C during which time samples were taken for carrying out analysis. After the fermentation was completed the matrix was pasteurised for 30 minutes at 80°C and cooled to room temperature.

Rheological measurements:
To demonstrate the effect of fermentation on the rheological properties of tomato paste a fermented sample was

compared with an unfermented sample in a standardised centrifugation test using serum layer development as an indicator. Thus 10 g of (un)fermented tomato paste was transferred each into a 16 x 100 mm culture tube and centrifuged for 0, 3 and 6 minutes at 540 g using a type Hettig 30F Universal centrifuge. After centrifugation the height of the serum layer was measured in mm:

Table 5. Effect of fermentation (as also indicated by viable cell count) on serum development in tomato paste at x 540 g force (dm = 8.59%).

Sample / centr. time	0 min	3 min	6 min	9 min	Viable count[1)]
Unfermented	< 1	18	22	26	< 10
Fermented	< 1	< 1	2	1	8×10^8

[1)] viable count in cfu/g before pasteurisation.

Example 6.

Same as example 5 above except that during fermentation samples have been analysed for serum development, pH and viable counts.

Table 6. Effect of fermentation time on tomato paste.

Fermentation time [hours]	Serum height [mm]	Viable count [cfu/g]	pH
0	35	2.0×10^6	6.5
4	33	4.0×10^6	6.5
8	25	1.4×10^8	6.1
11	2	1.9×10^8	5.4
24	2	6.8×10^8	4.3

Example 7.

The same procedure as described in example 6 was followed, however, now using Lactococcus lactis cremoris LAB 338 .

Result: serum layer in 10 g fermented product after 9 minutes at 540 g (for procedure see Examples 1 and 5) within range of 2 - 4 mm. Control sample (unfermented) same as in example 1.

Claims

1. A process for preparing a fruit product comprising the steps of:

i) breaking and/or crushing the fruit,

ii) adjusting the pH to 4.0 - 7.0, preferably to 5.5 - 6.5,

iii) sterilising and/or pasteurising the broken/crushed fruit,

iv) adding a culture of a Lactobacillus or Lactococcus-strain producing when growing an extracellular polysaccharide at a rate of at least 0.8 g/l at 20°C, a pH of 5.8 within 24 hours an a medium according to De Man, Rogosa and Sharpe (J. Appl. Bacteriol. 23: 130-135 (1960) followed by fermenting, and

v) pasteurising and/or sterilising the fermented fruit material.

2. Process according to claim 1, in which the fruit is from a plant of the family of the Solanaceae, preferably from Lycopersicum esculentum (=tomato), or any cultivar thereof.

3. Process according to claim 1 or 2, in which the Lactobacillus/Lactococcus is selected from the group comprising Lactobacillus sake 0-1 (CBS 532.92), Lactobacillus paracasei (LMG 9193t1 and LAB 97) and Lactococcus lactis cremoris (LAB 338) or a functional mutant thereof.

4. Process according to any of the claims 1-3, in which prior to pasteurisation/sterilisation deaeration of the broken and/or crushed fruit is effected.

5. Process according to any of the claims 1-5, in which prior to sterilisation sieving is effected.

6. Process according to any of the claims 1-5, in which fermentation is carried out at a temperature between 10 and 50°, preferably 20 and 40°C in the absence of supplied oxygen.

7. Process according to any of the claims 1-6, in which before adding the Lactobacillus or Lactococcus strain a saccharide, preferably sucrose is added to the broken and/or crushed fruit material.

8. Process according to any of the claims 1-7, in which the amount of sucrose in the broken/crushed fruit material is adjusted to a level of 15-25 g/kg fruit material.

9. Process according to any of the claims 1-8, in which the process from step ii) onwards is carried out under aseptic conditions.

10. A fermented food product obtainable by a process according to any of the preceding claims.

INTERNATIONAL SEARCH REPORT

	International Application No PCT/EP 97/02947

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L2/02 A23L2/84 A23L1/212 A23L1/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 308 064 A (KAGOME) 22 March 1989 cited in the application see claims; examples	1-10

A	DATABASE WPI Section Ch, Week 8240 Derwent Publications Ltd., London, GB; Class D13, AN 82-84396E XP002012722 & JP 57 138 370 A (KIRIN BREWERY KK) , 26 August 1982 see abstract	1-10

A	PATENT ABSTRACTS OF JAPAN vol. 013, no. 278 (C-611), 26 June 1989 & JP 01 074971 A (KAGOME KK), 20 March 1989, see abstract	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 September 1997	01. 10. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	**Authorized officer** Van Moer, A

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INTERNATIONAL SEARCH REPORT

	International Application No PCT/EP 97/02947

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 013, no. 278 (C-611), 26 June 1989 & JP 01 074972 A (KAGOME KK), 20 March 1989, see abstract -----	1-10

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
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EP 308064 A	22-03-89	JP 1074971 A	20-03-89
		JP 1980426 C	17-10-95
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